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THE ORIGIN OF THE BOUQUET ARRANGEMENT OF PROPHASE CHROMOSOMES IN *ALLIUM CEPA*

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Abstract

Using a denaturation-reannealing treatment to indicate the location of heterochromatin, it is shown that the bouquet arrangement of prophase chromosomes in *Allium cepa* is a consequence of the arrangement of chromosomes during interphase, which is itself derived from their arrangement at the previous telophase. The developmental implications of this are discussed.

Uittreksel

DIE OORSPRONG VAN DIE BOUQUET-RANGSKIKKING VAN DIE PROFASE CHROMOSOME IN *ALLIUM CEPA*

Deur gebruik te maak van 'n denaturasie-hervoeging behandeling om die posisie van die heterochromatien aan te dui, word dit getoon dat die bouquet-rangskikking in die profase chromosome in *Allium cepa* die gevolg is van die rangskikking van die chromosome gedurende die interfase, wat weer die gevolg van die rangskikking van die voorafgaande telofase is. Die ontwikkelingsimplikasies hiervan word bespreek.

Introduction

Over the past few years, considerable evidence has accumulated to indicate that the arrangement of chromosomes in interphase nuclei is non-random. Of particular value has been the application of denaturation-reannealing techniques which, by indicating the location of heterochromatin, offer new opportunities for following the positions of specific chromosome regions throughout the mitotic cycle.

Using one such technique, Mogford (1977) demonstrated that in root tip nuclei of the onion, *Allium cepa*, chromosomes maintain throughout interphase the arrangement established at the previous telophase. This finding has since been confirmed by other workers (Fussel, 1977; Ghosh and Roy, 1977).

Little attention has so far been devoted to the significance of the various types of chromosome arrangement observed during the prophase stages of mitosis. For example, it has long been known that, in many species, the chromosomes on their first appearance at prophase are present in a bouquet-like arrangement. The origin of this arrangement has remained unknown.

The present work was designed to investigate the origin of the bouquet arrangement of chromosomes in prophase nuclei of *A. cepa*, using the denaturation-reannealing technique previously used with success in determining the interphase arrangement of chromosomes in this species.

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MATERIAL AND METHODS

Investigations were performed on rapidly growing root tips of *Allium cepa* var. "White Lisbon". In some cases, the root tips were pre-treated by immersion in 0,5 % colchicine solution for three hours at room temperature, in order to accumulate metaphase stages; in other cases, the root tips were not pre-treated, these preparations being used for studies of the complete course of mitosis.

In either case, the material was fixed in 3:1 ethanol:glacial acetic acid overnight, and hydrolysed in 0,2N HCℓ at 60 °C for 2½ minutes. The meristems were squashed in 45 % acetic acid under an albumenised coverslip, the coverslip then being floated off in absolute alcohol, air-dried, and immersed in saturated barium hydroxide solution for 6 minutes at room temperature. The preparations were rinsed in running deionised water, incubated in 2 x saline sodium citrate buffer at 60 °C for 30 minutes, rinsed again in deionised water, air-dried and stained for 5 minutes in a 0,1 % alcoholic solution of the DNA-binding benzimidazole derivative Hoechst 33258.

The material was then rinsed in absolute alcohol, air-dried, mounted in 50 % glycerol and observed using a Zeiss fluorescence microscope with exciter filter BG12 and barrier filter 50. Photographs were taken using Kodak Tri-X Pan film.

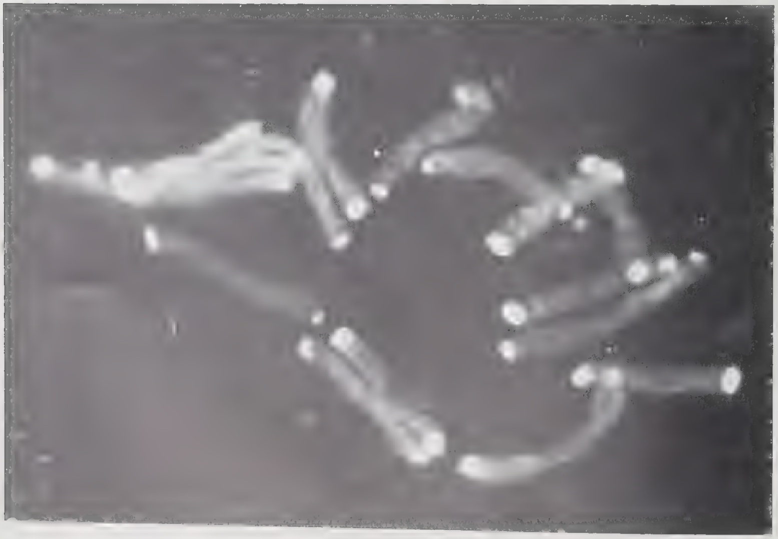


FIG. 1.
Metaphase complement of *A. cepa* var. "White Lisbon", prepared as described in text. Brightly fluorescent regions indicate location of heterochromatin.

RESULTS

The chromosome complement of *A. cepa* var. "White Lisbon" is shown in Figure 1. It may be seen that heterochromation is present on all the chromosomes and is almost exclusively terminal in position. The distribution of heterochromatin in this variety therefore appears identical to that in variety "Ailsa Craig" (Mogford, 1977).

The positions of the heterochromatic segments at the various stages of mitosis are illustrated in Figures 2–9. The typical bouquet arrangement of chromosomes referred to above is illustrated in Figure 3.

From comparisons of the positions of the heterochromatic segments at the various stages it may be seen that the bouquet arrangement of chromosomes is derived from the interphase arrangement of chromosomes described previously (Mogford, 1977) and illustrated in Figure 9. During interphase the chromosomes maintain the telophase arrangement of the previous mitosis, with telomeres grouped towards one side of the nucleus and centromeres towards the other.

The onset of prophase is marked by a contraction and loosening of the chromosomes (Fig. 2) to form the bouquet arrangement (Fig. 3), during which

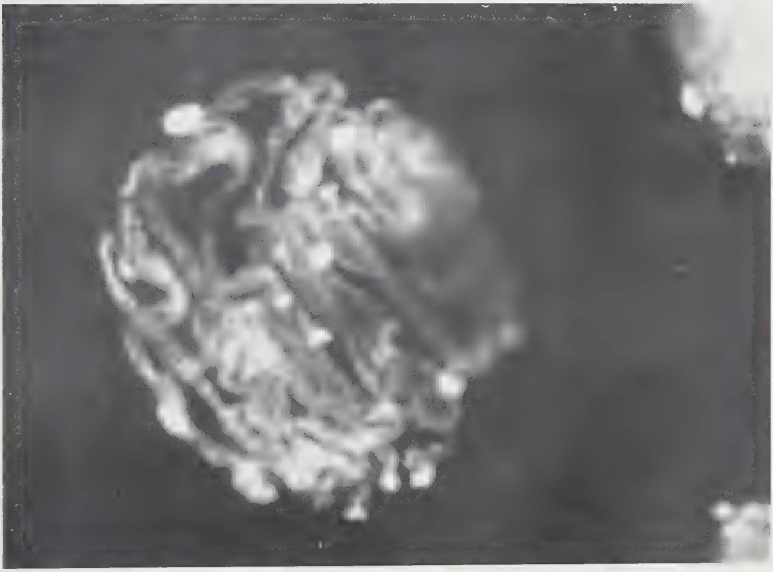


FIG. 2.
Early prophase.

FIGS 2–9.
The course of mitosis in *A. cepa* var. "White Lisbon".

stages the interphase polarisation of telomeres towards one side of the nucleus remains obvious. Continued contraction of the chromosomes occurs (Figs 4, 5) until the typical late prophase appearance is reached (Fig. 6), at which stage no obvious arrangement of the chromosomes remains. A regular arrangement, however, is evident at the succeeding metaphase and anaphase stages (Figs 7, 8), and by late telophase the daughter nuclei show the polarisation of heterochromatic segments typical of interphase (Fig. 9).

DISCUSSION

The results indicate that the bouquet arrangement of prophase chromosomes in *Allium cepa* is a direct consequence of their ordered arrangement in the interphase nuclei, which is in fact itself determined by their arrangement at the preceding telophase.

The maintenance of the ordered arrangement of chromosomes throughout interphase may be related to the occurrence of end-to-end fusions of the heterochromatic regions in this species (Mogford, 1977); however, the significance of such fusions remains obscure, despite confirmation of their existence by Fussel (1977).

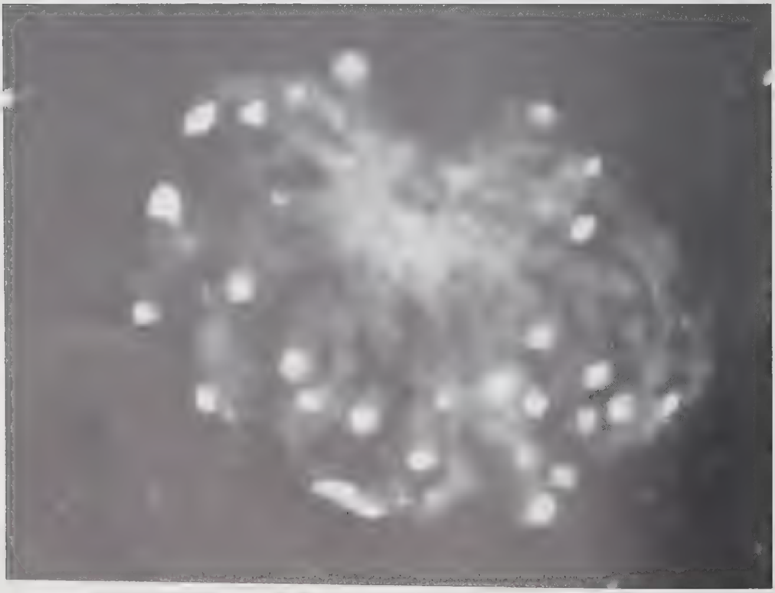
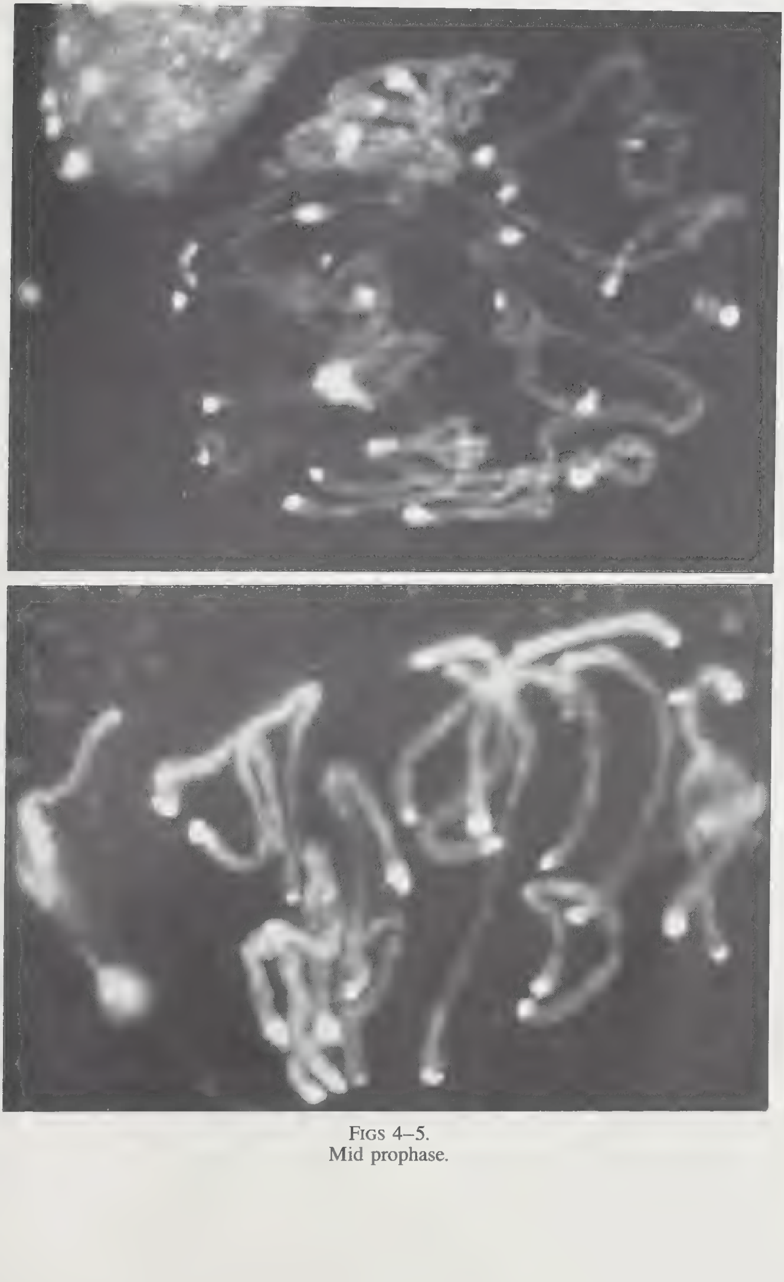


FIG. 3.
Prophase bouquet arrangement.



Figs 4–5.
Mid prophase.

An interesting consequence of the occurrence of fixed chromosome arrangements in interphase is the possibility that the plane of division of the nuclei may be determined by the orientation of the chromosomes. If this is the case, then the fixed arrangement of chromosomes, which extends at least from metaphase until the succeeding late prophase, must considerably reduce the number of planes of division available to the cell. If the fixed arrangement of chromosomes persists throughout late prophase as well, which is a possibility despite the haphazard arrangement of chromosomes at this stage revealed by the present technique, then the possible planes of cell division would be reduced still further.

Since the precise planes at which cell divisions occur is a crucial factor in development (Davidson, 1968), it is evident that further studies of mitotic chromosome arrangements may be of considerable significance in relation to the control of development.

REFERENCES

DAVIDSON, E. H., 1968. *Gene activity in early development*. New York: Academic Press.

FUSSEL, C. P., 1977. Interphase telomere associations in *Allium*. *Expl. Cell Res.* **110:** 111–117.

GHOSH, S., and ROY, S. C., 1977. Orientation of interphase chromosomes as detected by Giemsa C-bands. *Chromosoma* **61:** 49–55.

MOGFORD, D. J., 1977. Chromosome associations in onion root tip nuclei. *Jl S. Afr. Bot.* **43:** 97–102.

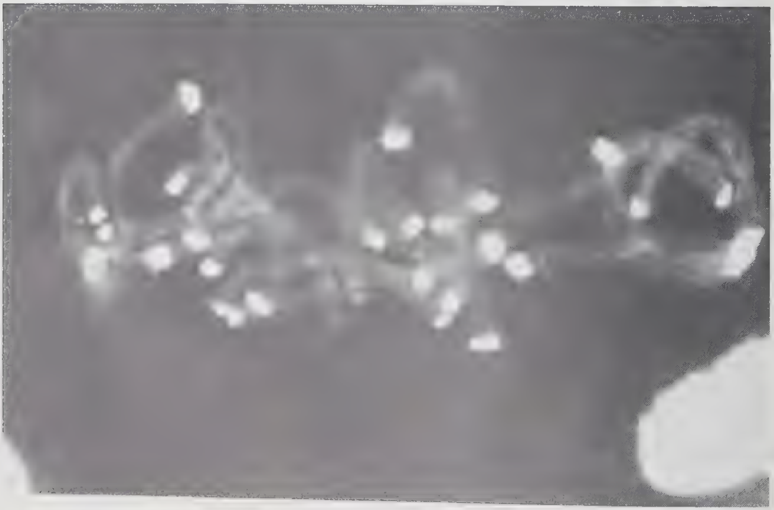


FIG. 6.
Late prophase.

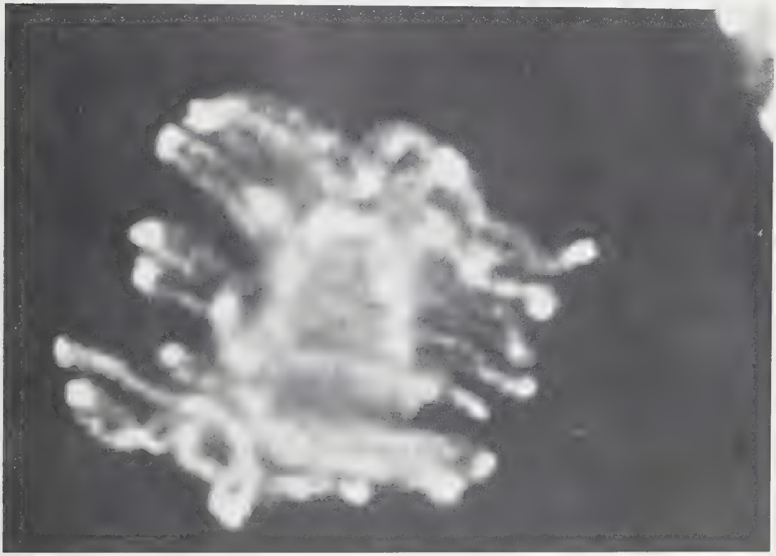


Fig. 7.
Metaphase.

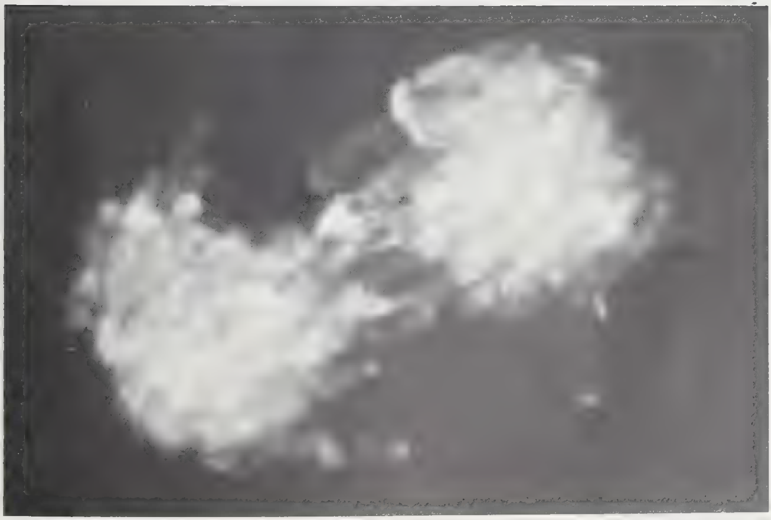


Fig. 8.
Anaphase.

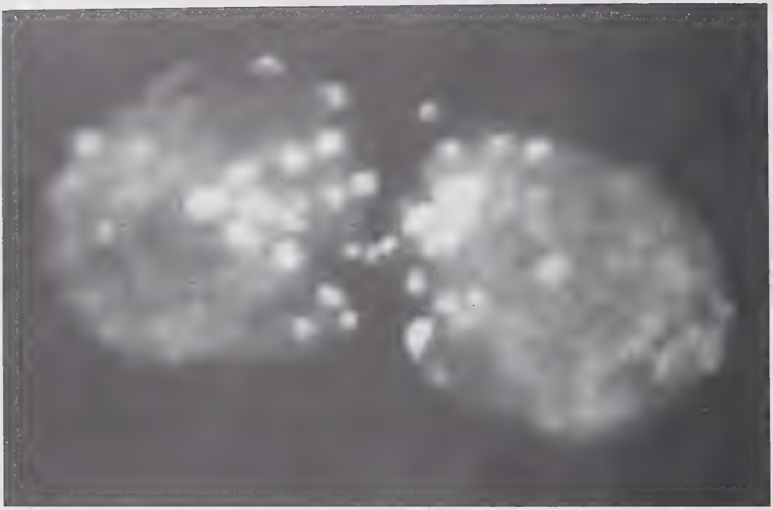


FIG. 9.
Late telophase.